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CITATION:

ABE, Kazunori ...[et al]. <Preliminary>Crystalline Features of Bacterial Cellulose Altered by Chemical Agents during Biosynthesis. Wood research : bulletin of the Wood Research Institute Kyoto University 1998, 85: 66-67

ISSUE DATE:

1998-09-30

URL:

http://hdl.handle.net/2433/53188

Preliminary

Crystalline Features of Bacterial Cellulose Altered by Chemical Agents during Biosynthesis*[1]

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(Received June 1, 1998)

Keywords: *Acetobacter*, bacterial cellulose, property enhancement

Bacterial cellulose consists of flat ribbon-shaped microfibrils. Each ribbon usually has a size of approximately 100 nm wide and 3 to 8 nm thick, and thought to be composed of a number of sub-elementary units. The loci of the biosynthesis of these sub-fibrils are found on the outer surface of bacterium as a linearly ordered array of putative synthases, Terminal Complexes. Each TC produces nanometer size sub-elements, and every three elements are thought to be crystallized into a microfibril by hydrogen bonding. Several microfibrils are eventually aggregated into one single ribbon that can be observed by conventional electron microscopy. These ribbons are not reassembled into the secondary structure nor embedded in encrusting compounds as in the higher plant cell walls.

This microfibrillar nature of bacterial cellulose brings a number of superior characteristics, and one of the good example would be the successful application as an acoustic diaphragm with high Young's modulus and loss tangent. Improvement of the physical properties of such materials is of great importance as it is directly connected to the structure of cellulose and in this sense one of the most interesting approach is to increase the size of microfibrils. Obviously, for instance, the wider the microfibril dimension becomes the better elastic moduli would result.

Recently, Ishihara and Yamanaka[1)] found that the addition of several reagents induced the modification of cell morphology of bacteria. Two chemicals were found to elongate cells by inhibiting the separation after the cell division. Another reagent, a reducing agent, effectively shortened the cell on the contrary. In this study, therefore, the changes of microfibrillar morphology induced by such chemical reagents were investigated.

Microfibrils were observed by electron microscopy after mild homogenization of the

*[1] A part of this work was presented at the 47th Annual Meeting of the Japan Wood Research Society in Kochi (April, 1997).

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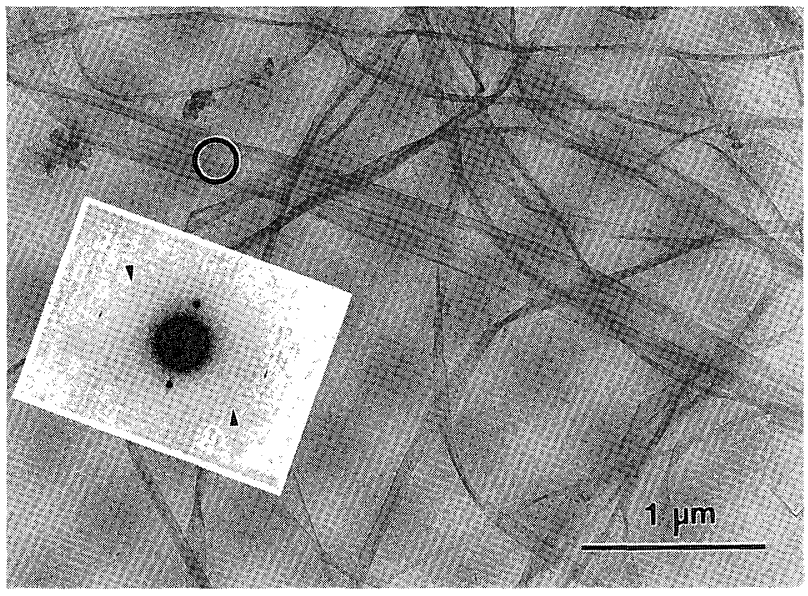

Fig. 1. Typical electron micrograph of altered microfibrils of *Acetobacter aceti*. In this case, the cell lengths are about 2-3 times longer than the control. Arrowheads: typical triclininc ($1\bar{0}3$) diffraction spots.

bacterial mat. Interestingly, the width of ribbons increased as the bacterial cell length increased. In some cases the treatment allowed to produce twice as large ribbons on an average. The distribution of the width was, however, rather heterogeneous as in Fig. 1: some ribbons are as wide as diffracting the typical triclininc diffraction spots of ($1\bar{0}3$)[2] but some remain to be intact.

Several crystallographic features have been also investigated, such as relative crystallinity index, uniplaner orientation behavior, crystallites size from X-ray diffractometry, the fractional ratio of I_α from FT-IR spectroscopy. Although the crystallite size deduced from a conventional Sherrer equation showed little change but the relative crystallinity, uniplanar orientation behavior of 0.62 nm, and I_α fraction tended to increase in relation to the increase of the cell length. This suggests that the chemical treatment is less influential on the formation of each sub-fibril (crystalline core) but affected the aggregation of sub-fibrils to a higher-order structure such as microfibrils and ribbons.

In order to elucidate the possible causes for treating by chemical reagents, the arrangement of terminal complexes was observed by freeze-fracture deep-etching technique. Although the finding is considered as preliminary, the chemical reagent seems to influence not only the size of the bacterial cells but also the number of TCs as well as their two-dimensional arrangement.

References

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